American Journal of Epidemiology

Vol. 160, No. 4
Printed in U.S.A.
DOI: 10.1093/aje/kwh219

ORIGINAL CONTRIBUTIONS

Poliovirus Vaccination during Pregnancy, Maternal Seroconversion to Simian Virus 40, and Risk of Childhood Cancer

E. A. Engels[1], J. Chen[1], R. P. Viscidi[2], K. V. Shah[3], R. W. Daniel[3], N. Chatterjee[1], and M. A. Klebanoff[4]

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD.
[2] Department of Pediatrics, School of Medicine, Johns Hopkins University, Baltimore, MD.
[3] Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.
[4] Division of Epidemiology, Statistics, and Prevention Research, National Institute of Child Health and Human Development, Rockville, MD.

Received for publication October 20, 2003; accepted for publication March 2, 2004.

Before 1963, poliovirus vaccine produced in the United States was contaminated with simian virus 40 (SV40), which causes cancer in animals. To examine whether early-life SV40 infection can cause human cancer, the authors studied 54,796 children enrolled in the US-based Collaborative Perinatal Project (CPP) in 1959–1966, 52 of whom developed cancer by their eighth birthday. Those children whose mothers had received pre-1963 poliovirus vaccine during pregnancy (22.5% of the children) had an increased incidence of neural tumors (hazard ratio = 2.6, 95% confidence interval: 1.0, 6.7; 18 cases) and hematologic malignancies (hazard ratio = 2.8, 95% confidence interval: 1.2, 6.4; 22 cases). For 50 CPP children with cancer and 200 CPP control children, the authors tested paired maternal serum samples from pregnancy for SV40 antibodies using a virus-like particle enzyme immunoassay and a plaque neutralization assay. Overall, mothers exhibited infrequent, low-level SV40 antibody reactivity, and only six case mothers seroconverted by either assay. Using the two SV40 assays, maternal SV40 seroconversion during pregnancy was not consistently related to children's case/control status or mothers' receipt of pre-1963 vaccine. The authors conclude that an increased cancer risk in CPP children whose mothers received pre-1963 poliovirus vaccine was unlikely to have been due to SV40 infection transmitted from mothers to their children.

BK virus; brain neoplasms; child; leukemia; neoplasms; poliovirus; poliovirus vaccines; simian virus 40

Abbreviations: CI, confidence interval; OR_{SA}, sampling-adjusted odds ratio; SV40, simian virus 40; VLP, virus-like particle.

Simian virus 40 (SV40), a macaque polyomavirus, contaminated 10–30 percent of doses of poliovirus vaccine used in the United States from 1955 through 1962 (1). This contamination arose because, before the discovery of SV40 in 1960 and subsequent changes in vaccine manufacture, poliovirus vaccines were produced in monkey tissue harboring SV40. In the United States, SV40-contaminated inactivated poliovirus vaccine was given parenterally to tens of millions of people, and some also received SV40-contaminated oral poliovirus vaccine (1). These exposures have raised concern, because SV40 causes malignancies in laboratory rodents (2). Some researchers, though not all, have identified SV40 DNA sequences in a variety of human tumors, including pediatric brain tumors (3, 4). Given conflicting data, the question of whether SV40 causes cancer in humans remains controversial (see review by the Institute of Medicine (2)).

During the period when poliovirus vaccines were contaminated with live SV40, inactivated poliovirus vaccine was

Correspondence to Dr. Eric A. Engels, Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., EPS 8010, Rockville, MD 20892 (e-mail: engelse@exchange.nih.gov).

frequently administered to pregnant women in the United States. Conceivably, mothers acquiring SV40 infection as a result of vaccination during pregnancy could have transmitted the virus to their children, either in utero or shortly after birth. In laboratory animals, SV40 acquired during the newborn period is especially tumorigenic (5). Nonetheless, large-scale follow-up studies of children who received SV40-contaminated poliovirus vaccine as young children (even as neonates) have not revealed them to be at increased cancer risk (6–8).

Heinonen et al. (9) previously examined the possible association between maternal poliovirus vaccination during pregnancy and childhood cancer, using data on children under age 4 years from the Collaborative Perinatal Project (described in more detail below). These investigators found a strong relation between maternal poliovirus vaccination and risk of neural tumors (relative risk = 12; $p < 0.01$). They also noted a nonsignificant excess risk of leukemia in children whose mothers had received poliovirus vaccine during pregnancy (relative risk = 1.8; p value not reported). However, these results were based on only eight children with neural tumors and eight children with leukemia. Furthermore, Heinonen et al. considered all exposures to inactivated poliovirus vaccine during pregnancy as the relevant risk factor for childhood cancer, although only pre-1963 inactivated poliovirus vaccine contained SV40 (1). Subsequently, Rosa et al. (10) found SV40 antibodies in only 9 percent of the mothers of the cancer cases studied by Heinonen et al. Thus, while Heinonen et al.'s study is the only cohort study to have found a relation between exposure to early poliovirus vaccine and cancer, the small number of cancer cases and the lack of serologic evidence of SV40 infection in the mothers complicate interpretation of its findings.

In the present study, we extended the prior work by Heinonen et al. and Rosa et al. We examined cancer incidence among children in the Collaborative Perinatal Project up to their eighth birthday and included 52 children with cancer, more than twice as many as were evaluated previously (9). Additionally, we separately considered maternal exposures to pre-1963 poliovirus vaccine (both inactivated poliovirus vaccine and oral poliovirus vaccine, both of which were contaminated with SV40) and poliovirus vaccine manufactured in or after 1963 (which was free of SV40). Finally, we measured SV40 serostatus in a case-control study within the Collaborative Perinatal Project, to examine whether SV40 infection could reasonably account for associations between maternal receipt of poliovirus vaccine and subsequent cancer in children.

MATERIALS AND METHODS

Cohort description, vaccine exposures, and cancer outcomes

The Collaborative Perinatal Project, a cohort study designed to evaluate risk factors for childhood neurodevelopmental disorders, enrolled pregnant women and their subsequently born children at 12 US university medical centers in 1959–1966 (11). The cohort comprised 54,796 children born to 44,621 mothers. Enrolled mothers had study visits scheduled as an integral part of their prenatal and subsequent medical care. At their first visit (median of 20 weeks' gestation), mothers provided a detailed medical history, including history of vaccinations, and a blood sample. At later visits during and after pregnancy, mothers provided interval histories and additional blood samples. Children were seen at 4-month intervals during their first year and at 4 years and 7–8 years of age, and mothers were interviewed annually (12). After being informed regarding the purpose of the Collaborative Perinatal Project, participating mothers provided verbal consent. The present investigation was approved by a National Cancer Institute institutional review board.

At study visits made during pregnancy, information on receipt of poliovirus vaccine was obtained from interview of mothers and a review of clinic records. Poliovirus vaccine type was classified as inactivated, oral, or unspecified. Information on the date of the last menstrual period and the lunar month in which vaccination occurred was used to date vaccine exposures (classified for analysis as pre-1963 vs. 1963+).

Malignancies arising in Project children up to their eighth birthday had previously been identified in several ways (13). First, study identification numbers of children with cancer were obtained through a previous study of childhood cancer in this cohort (14). Second, potential cancer cases were identified in a review of records of all children without life-threatening anomalies who weighed at least 1,500 g at birth and who died after the first week of life. Third, Project diagnostic summary forms, completed at 1 and 7 years of age, were reviewed. Cases were included only if cancer was confirmed by a medical record summary that provided a histologic diagnosis, a clinical course (including treatment) consistent with a cancer diagnosis, or both.

Statistical methods for cohort analysis

We calculated the incidence (number of events per 100,000 person-years) of all cancers together and, separately, the incidence of neural tumors, hematologic malignancies, and miscellaneous tumors (see table 1 for classification). Children were considered to be under follow-up through their last examination (median duration of follow-up, 7.3 years; interquartile range, 6.0–8.0 years). We calculated incidence separately for children with maternal receipt during pregnancy of pre-1963 poliovirus vaccine (possibly contaminated with SV40), children with maternal receipt during pregnancy of only 1963+ poliovirus vaccine (SV40-free), and children with no maternal receipt of poliovirus vaccine during pregnancy. For each cancer outcome, we plotted Kaplan-Meier curves for these three strata. Proportional hazards regression was used to derive vaccine-associated hazard ratios for these cancer outcomes and to adjust for possible confounding by demographic characteristics. These analyses used a method of Wei et al. (15) that accounted for correlated outcomes in children with the same mother.

TABLE 1. Malignancies observed during follow-up among 54,796 children aged 0–7 years enrolled in the Collaborative Perinatal Project in 1959–1966

Malignancy	All children (ages 0–7 years)		Children aged 0–3 years				Children aged 4–7 years	
			Included in study by Heinonen et al. (9)		Not included in study by Heinonen et al. (9)			
	No. of cases	No. exposed*	No. of cases	No. exposed*	No. of cases	No. exposed*	No. of cases	No. exposed*
Neural tumors	18	8	6	4	6	0	6	4
Neuroblastoma	7	5	2	2	1	0	4	3
Astrocytoma/glioma	5	1	2	0	1	0	2	1
Retinoblastoma	3	1	1	1	2	0	0	0
Ependymoma	1	0	0	0	1	0	0	0
Medulloblastoma	1	1	1	1	0	0	0	0
Not specified	1	0	0	0	1	0	0	0
Hematologic malignancies	22	10	8	4	7	3	7	3
Leukemia	17	7	7	3	5	3	5	1
Non-Hodgkin's lymphoma	4	2	1	1	2	0	1	1
Hodgkin's disease	1	1	0	0	0	0	1	1
Miscellaneous tumors	12	3	4	2	3	0	5	1
Wilms' tumor	7	3	3	2	2	0	2	1
Hepatoblastoma	2	0	1	0	1	0	0	0
Rhabdomyosarcoma	2	0	0	0	0	0	2	0
Fibrosarcoma	1	0	0	0	0	0	1	0
All malignancies	52	21	18	10	16	3	18	8

* Number of cases whose mothers received pre-1963 poliovirus vaccine during pregnancy.

Case-control study

We also conducted a case-control study based within this cohort to determine whether SV40 seroconversions during pregnancy were more common in mothers of cases than in mothers of controls and whether SV40 seroconversions were related to receipt of pre-1963 poliovirus vaccine. Cases (n = 50) were those children who developed a malignancy during follow-up and for whom two maternal serum samples were available from the pregnancy with the study child (the index pregnancy). Similarly, we selected 200 controls who did not develop malignancy during completed follow-up (i.e., "cumulative" control sampling (16)) and for whom two stored serum samples were available from the index pregnancy (see description of sampling below). For some case and control mothers, the second serum sample was obtained after delivery.

The primary measure of children's SV40 exposure was maternal SV40 seroconversion during pregnancy, which could be measured for all children with paired maternal serum samples. Nonetheless, we considered that poliovirus vaccination was the most likely route by which mothers could acquire SV40 during pregnancy, so we additionally classified their paired serum samples as "informative" or "uninformative" according to the likelihood of observing SV40 seroconversion related to vaccination—that is, mothers' paired serum samples were considered informative if they bracketed vaccination in an appropriate manner. Specifically, we allowed for a lag of 6 weeks for the development of SV40 antibodies following SV40 infection. Thus, paired serum samples were considered informative for SV40 seroconversion if at least one dose of poliovirus vaccine was administered during the window from 6 weeks prior to the first serum sample to 6 weeks prior to the second serum sample.

By design, the 200 controls were randomly sampled in varying proportions from strata defined by maternal vaccination status and informativeness (table 2). This two-stage sampling strategy allowed us to increase statistical efficiency (i.e., we maximized the expected number of seroconverters by oversampling informative mothers and those who received pre-1963 inactivated poliovirus vaccine) and to have sufficient numbers of control mothers with various vaccine exposures to examine the relation between vaccination and SV40 seroconversion (16). Because of this sampling strategy, crude seroconversion rates and corresponding odds ratios do not reflect the underlying parameters. Therefore, as described below, we incorporated information on the sampling fractions from control strata into the analyses to obtain valid comparisons of seroconversion rates across groups.

TABLE 2. Characterization of mothers of cancer cases (n = 50) and controls (n = 200) enrolled in the Collaborative Perinatal Project in 1959–1966 who were selected for a case-control study of poliovirus vaccine

	All mothers			Mothers with informative serum samples*		
	No. selected	No. available	Mean window (weeks)†	No. selected	No. available	Mean window (weeks)†
Case mothers	50	50	24.9	21	21	24.9
Received pre-1963 vaccine	20	20	23.1	16	16	25.1
Pre-1963 IPV‡	20	20	23.1	16	16	25.1
Pre-1963 OPV‡	0	0		0	0	
Did not receive pre-1963 vaccine	30	30	26.1	5	5	24.3
1963+ IPV	1	1	29.6	1	1	29.6
1963+ OPV	5	5	24.9	4	4	22.9
No vaccine	24	24	26.2	NA‡	NA	NA
Control mothers§	200	48,745	22.0	131	15,257	23.6
Received pre-1963 vaccine	100	10,771	20.7	82	8,389	22.3
Pre-1963 IPV	90	10,187	20.6	72	8,026	22.5
Pre-1963 OPV	10	584	21.3	10	363	21.3
Did not receive pre-1963 vaccine	100	37,974	23.3	49	6,868	25.6
1963+ IPV	45	6,583	26.0	45	5,848	26.0
1963+ OPV	5	2,242	18.1	4	1,020	21.7
No vaccine	50	29,149	21.4	NA	NA	NA

* Informative serum pairs were those in which mothers received at least one dose of poliovirus vaccine in the window defined by the serum dates.

† The window was defined by the paired maternal serum samples evaluated for polyomavirus antibodies. Specifically, the window was the period from 6 weeks before the first serum sample to 6 weeks before the second serum sample; see Materials and Methods.

‡ IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; NA, not applicable.

§ A total of 122 control mothers who received "unspecified poliovirus vaccine" (not specified as inactivated poliovirus vaccine or oral poliovirus vaccine) during pregnancy were excluded from potential sampling.

Laboratory methods

Antibody testing was performed on paired maternal serum samples (previously stored at –20°C) for the earliest and latest samples from each mother's pregnancy. To mask specimens with respect to case/control status and pairing, we relabeled samples and randomly sorted them prior to shipment to testing laboratories.

Sera were tested for SV40 antibodies using a previously described virus-like particle (VLP) enzyme immunoassay (17). Briefly, SV40 VLPs are empty capsids, generated by spontaneous self-assembly of the major capsid protein VP1, that retain many immunologic properties of native virions. In rhesus macaques, the SV40 VLP assay previously exhibited 100 percent sensitivity and 100 percent specificity with respect to a plaque neutralization assay (plaque assay) (17). For the present study, we included control serum samples from macaques that were seropositive (n = 29) or seronegative (n = 10) on the plaque assay. In humans, some reactivity measured by the SV40 VLP assay may represent cross-reactivity to the human polyomavirus BK (17–19). Therefore, we also tested all specimens for antibodies to BK virus using an analogous BK virus VLP enzyme immunoassay (17). All specimens in both VLP assays were measured in duplicate, and we used the geometric mean of the duplicates in the analyses. SV40 VLP seropositivity was determined using a cutoff absorbance of 0.10 optical density units, obtained from an inspection of the histogram of absorbance results and similar to that used previously (19).

Additionally, we performed a plaque assay for SV40 neutralizing antibodies (20). Sera initially positive (i.e., 80 percent reduction of plaque counts as compared with control wells) at a dilution of 1:10 were titrated further at 1:40 and 1:160 dilutions. Titers were missing for 18 specimens because of fungal contamination of the assay plates. With each batch of approximately 100 serum samples, we included diluted serum from an SV40-infected macaque. This positive control sample inhibited plaque formation consistently at 1:500 and 1:5,000 dilutions and variably at a 1:50,000 dilution.

Statistical methods for case-control analysis

Using paired SV40 VLP results, we classified mothers as seroconverters (negative on the first specimen, positive on the second specimen), seroprevalent (positive, positive), seronegative (negative, negative), or seroreverters (positive, negative). We defined plaque assay seroconversion similarly, considering a titer of 1:40 a positive result. Women whose seroconversion status could not be classified because of missing titers were excluded from analysis. As alternative definitions for seroconversion, we considered other cutoff points (0.08 optical density units for the VLP assay; titers of 1:10 and 1:160 for the plaque assay) and a fourfold increase in titer (plaque assay), but these did not produce qualitatively different results (not shown).

We hypothesized that SV40 seroconversion during pregnancy (i.e., new SV40 infection) would be associated with the highest cancer risk in children, although we considered that seroprevalent maternal status might also convey risk. To address this question, we fitted a logistic regression model to assess whether maternal SV40 seroconversion status (seroconverter vs. seroprevalent vs. other; independent variable) was related to risk of childhood cancer (case/control status; dependent variable). We used software developed by Breslow and Chatterjee (21) to estimate parameters and their variances with a pseudolikelihood method that adjusted for the sampling scheme described above (22).

We also fitted a logistic regression model to assess whether, among control mothers, SV40 seroconversion (dependent variable) was related to vaccination status (receipt of pre-1963 inactivated poliovirus vaccine vs. other poliovirus vaccine/no vaccine; independent variable). We used as weights the sampling fractions from strata defined by vaccination and informativeness. Asymptotic variance estimates were obtained (23).

RESULTS

Cohort description

Of 54,796 children in the Collaborative Perinatal Project, 27,857 (50.8 percent) were male, and 25,148 (45.9 percent) were White. The median age of mothers at delivery was 23.8 years (interquartile range, 20.5–28.6).

Overall, 21,649 children (39.5 percent) had mothers who received poliovirus vaccine during pregnancy: 12,334 children (22.5 percent) had mothers who received pre-1963 poliovirus vaccine and 9,315 (17.0 percent) had mothers who received only 1963+ poliovirus vaccine. Maternal receipt of inactivated poliovirus vaccine during pregnancy declined over time (i.e., 38–51 percent for children born in 1959–1962 vs. 25–33 percent for children born in 1963–1966). Maternal receipt of oral poliovirus vaccine during pregnancy was rare (<4 percent) for children born before 1963 and peaked at 19 percent for those born in 1964. Additionally, few mothers (<1 percent) received poliovirus vaccine of unspecified type. Among mothers who received pre-1963 poliovirus vaccine during pregnancy, the mean number of doses was 1.3.

White children were more likely to have mothers who received poliovirus vaccine during pregnancy than were non-White children (48.6 percent vs. 31.8 percent; $p < 0.0001$). Males were slightly less likely to have vaccinated mothers than were females (39.1 percent vs. 39.9 percent; $p = 0.05$). In addition, vaccinated mothers were older than unvaccinated mothers (mean age = 25.5 years vs. 24.7 years; $p < 0.0001$).

Association between maternal receipt of poliovirus vaccine and malignancies in children

Fifty-two malignancies were identified during follow-up (incidence = 15 per 100,000 person-years). These included 18 neural tumors, 22 hematologic malignancies, and 12 miscellaneous tumors (table 1). Overall, we included 16 cancers diagnosed at ages 0–3 years that were not previously identified by Heinonen et al. (9). In addition, we excluded six cases included in Heinonen et al.'s report: two microscopic neuroblastomas diagnosed only at autopsy (readily confused with a normally developing adrenal gland) (24), one ovotestis mistakenly classified as a tumor, and three Wilms' tumors that we could not identify (all three were reportedly found in children whose mothers had not received poliovirus vaccine during pregnancy). One case of leukemia was reclassified as a case of non-Hodgkin's lymphoma.

Children whose mothers had received pre-1963 poliovirus vaccine during pregnancy had the highest overall risk of cancer, while children whose mothers had received only 1963+ poliovirus vaccine or no vaccine had similar risks (figure 1, panel A). This difference in risk between children whose mothers had received pre-1963 vaccine and those whose mothers had not was significant (hazard ratio = 2.3, $p = 0.004$; table 3). Similar patterns were observed specifically for neural tumors and hematologic malignancies (figure 1, panels B and C), with significantly elevated risks being present for children whose mothers had received pre-1963 poliovirus vaccine (hazard ratio = 2.6 ($p = 0.04$) and hazard ratio = 2.8 ($p = 0.02$), respectively; table 3). Among neural tumors, there were sufficient numbers of cases only of neuroblastoma to examine separately the association with maternal receipt of pre-1963 vaccine (hazard ratio = 8.2, 95 percent confidence interval (CI): 1.6, 43; $p = 0.01$). The risk of miscellaneous tumors did not differ across groups (figure 1, panel D; table 3). These relations did not change after adjustment for sex, race, and maternal age (table 3).

Subjects included in the case-control study

Paired maternal serum samples were available for 50 cases (96 percent). We selected 200 of 48,745 eligible control mothers, of whom 100 (50 percent) had received pre-1963 poliovirus vaccine during pregnancy (90 had received pre-1963 inactivated poliovirus vaccine). The remaining 100 control mothers either had received only 1963+ poliovirus vaccine ($n = 50$) or had not received poliovirus vaccine ($n = 50$).

Among case and control mothers who received poliovirus vaccine during pregnancy, the proportions with informative paired serum samples (i.e., serum samples that bracketed poliovirus vaccination) were similar (81 percent vs. 87 percent). In addition, the durations of windows defined by

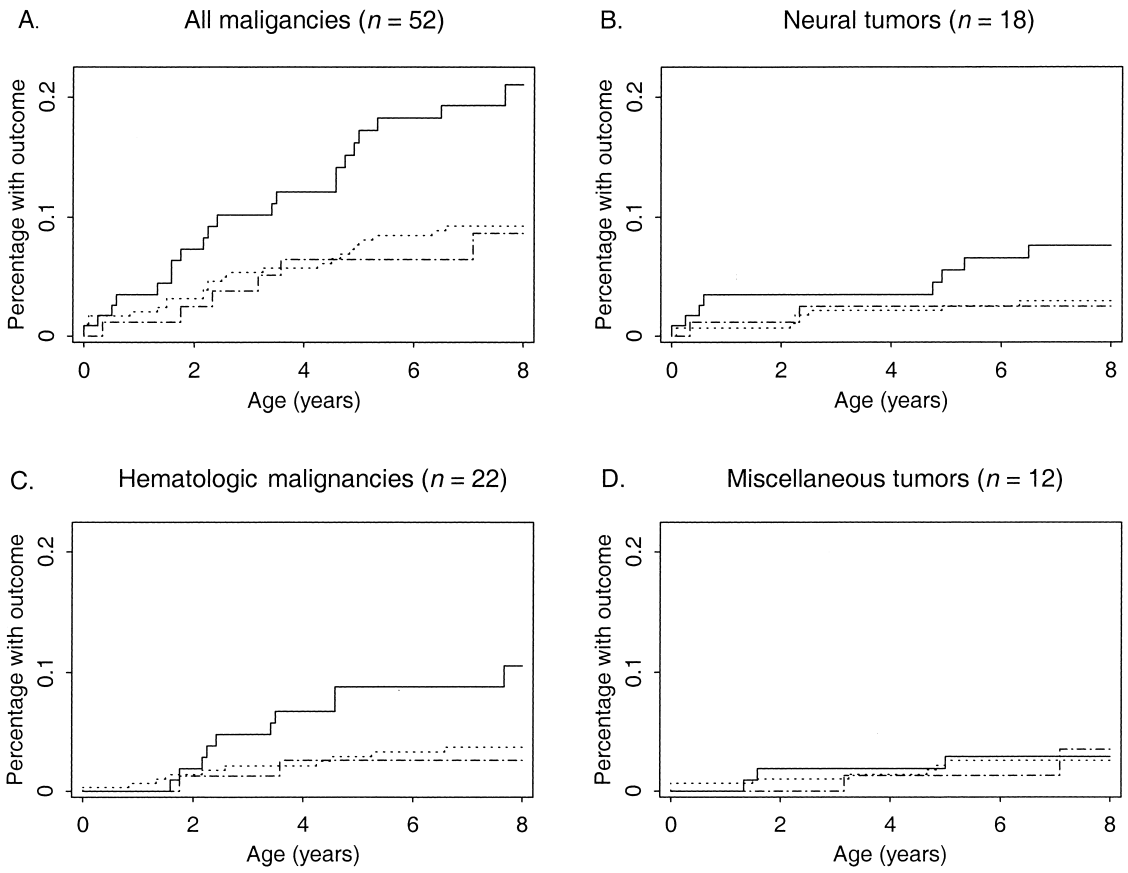

FIGURE 1. Incidence of cancer among children aged 0–7 years enrolled in the Collaborative Perinatal Project in 1959–1966, as a function of maternal receipt of poliovirus vaccine during pregnancy. Panels A–D present data for all malignancies combined (panel A), neural tumors (panel B), hematologic malignancies (panel C), and miscellaneous tumors (panel D). Children were classified according to their mothers' vaccination status during pregnancy: solid line, receipt of pre-1963 poliovirus vaccine; dashed line, receipt of poliovirus vaccine only in or after 1963; dotted line, no poliovirus vaccine exposure. Table 3 provides corresponding hazard ratios and results of significance tests (see text).

paired serum samples were similar in case and control mothers (mean of 24.9 weeks vs. 22.0 weeks; table 2).

SV40 antibody results

SV40 VLP assay results for 500 serum samples (i.e., paired specimens from 250 case and control mothers) are shown in figure 2, panel A. SV40 VLP results were bimodally distributed, with a trough at an absorbance of 0.10. Using this value as a cutoff, 82 serum samples (16 percent) were considered SV40 VLP-seropositive. Using the same cutoff, the SV40 VLP assay identified 28 of 29 SV40 plaque assay-seropositive monkeys (97 percent) as VLP-seropositive, while 10 of 10 plaque assay-seronegative monkeys (100 percent) were VLP-seronegative.

Ninety-nine serum samples (20 percent) were positive on the plaque assay. Plaque assay antibody titers were available for 81 samples: 30 (37 percent) were positive at 1:10, 33 (41 percent) were positive at 1:40, and 18 (22 percent) were positive at 1:160. SV40 plaque assay titers were modestly correlated with SV40 VLP results (Spearman correlation = 0.36; $p < 0.0001$).

SV40 seroconversion in mothers of cases and controls

Using the SV40 VLP assay (table 4), SV40 seroconversions were seen in four case mothers (8 percent) and six control mothers (3 percent). Accordingly, case mothers were more likely to seroconvert during pregnancy than control mothers (sampling-adjusted odds ratio (OR_{SA}) = 4.0, 95 percent CI: 1.0, 15.7). Case mothers and control mothers were equally likely to be SV40-seroprevalent at the start of pregnancy (12 percent vs. 13 percent; $OR_{SA} = 0.8$, 95 percent CI: 0.3, 2.1).

Using the plaque assay, SV40 seroconversion was evaluable for 233 mothers (table 4). Seroconversions occurred in three case mothers and eight control mothers (7 percent vs. 4 percent; $OR_{SA} = 1.5$, 95 percent CI: 0.3, 6.3), while two case mothers and 13 control mothers were seroprevalent (4 percent vs. 7 percent; $OR_{SA} = 0.3$, 95 percent CI: 0.1, 1.7). In

TABLE 3. Association between maternal receipt of poliovirus vaccine during pregnancy and childhood cancer among children enrolled in the Collaborative Perinatal Project in 1959–1966

Outcome and maternal vaccination status during pregnancy	No. of events	Incidence per 10^5 person-years	Unadjusted HR*	95% CI*	*p* value†	Adjusted HR‡	95% CI	*p* value†
All cancers								
No vaccine	25	12	1.0§			1.0§		
1963+ poliovirus vaccine	6	11						
Pre-1963 poliovirus vaccine	21	27	2.3	1.3, 3.9	0.004	2.1	1.2, 3.7	0.008
Neural tumors								
No vaccine	8	4	1.0§			1.0§		
1963+ poliovirus vaccine	2	4						
Pre-1963 poliovirus vaccine	8	10	2.6	1.0, 6.7	0.04	2.5	1.0, 6.3	0.06
Hematologic malignancies								
No vaccine	10	5	1.0§			1.0§		
1963+ poliovirus vaccine	2	4						
Pre-1963 poliovirus vaccine	10	13	2.8	1.2, 6.4	0.02	2.5	1.1, 5.6	0.03
Miscellaneous tumors								
No vaccine	7	3	1.0§			1.0§		
1963+ poliovirus vaccine	2	4						
Pre-1963 poliovirus vaccine	3	4	1.1	0.3, 4.1	0.88	1.2	0.3, 4.6	0.82

* HR, hazard ratio; CI, confidence interval.
† *p* values are two-sided and were calculated using a Wald test.
‡ Adjusted for sex, race (White vs. non-White), and mother's age at delivery (approximate tertiles: <21, 21–24, or ≥25 years).
§ Reference category.

total, only six case mothers seroconverted to SV40 during pregnancy, according to the VLP or plaque assay (table 5).

Receipt of pre-1963 inactivated poliovirus vaccine was associated with SV40 seroconversion according to the VLP assay but not the plaque assay. Specifically, among control mothers, VLP assay seroconversion was seen in four (4 percent) who received pre-1963 inactivated poliovirus vaccine and two (2 percent) who did not (OR_{SA} = 7.5, 95 percent CI: 1.4, 42). With the plaque assay, SV40 seroconversion was observed in four control mothers (5 percent) who received pre-1963 inactivated poliovirus vaccine and four (4 percent) who did not (OR_{SA} = 0.9, 95 percent CI: 0.2, 3.8).

BK virus antibody results

BK virus VLP reactivity was generally much stronger than SV40 VLP reactivity, and results were less clearly bimodal (figure 2, panel B). BK virus results were moderately correlated with SV40 VLP results (Spearman correlation = 0.40; $p < 0.0001$) and less strongly correlated with SV40 plaque assay titers (Spearman correlation = 0.20; $p < 0.0001$).

When we used the same cutoff value as that used for the SV40 VLP assay (absorbance = 0.10), 377 samples (75 percent) were seropositive for BK virus. Most mothers (80 percent of cases, 70 percent of controls) were BK virus-seroprevalent at the start of pregnancy. BK virus seroconversions were observed in two case mothers (4 percent) and seven control mothers (4 percent).

DISCUSSION

Our analysis of data from the Collaborative Perinatal Project identified significant relations between maternal receipt of poliovirus vaccine during pregnancy and risk of neural tumors and hematologic malignancies in subsequently born children (figure 1). These associations were limited to poliovirus vaccine administered prior to 1963, when perhaps 10–30 percent of doses of inactivated poliovirus vaccine contained live SV40 as a contaminant (1). Notably, however, in our accompanying case-control study, we found that mothers of Collaborative Perinatal Project cancer cases and controls exhibited only infrequent and low-level antibody reactivity to SV40. Indeed, maternal SV40 seroconversion during pregnancy was rare, even for cancer cases (table 5). As we discuss below, these findings do not support a role for SV40 in inducing malignancy in these children.

In our cohort analysis, the observed association between maternal poliovirus vaccination and neural tumors (hazard ratio = 2.6) was markedly attenuated compared with that reported previously by Heinonen et al. (relative risk = 12)

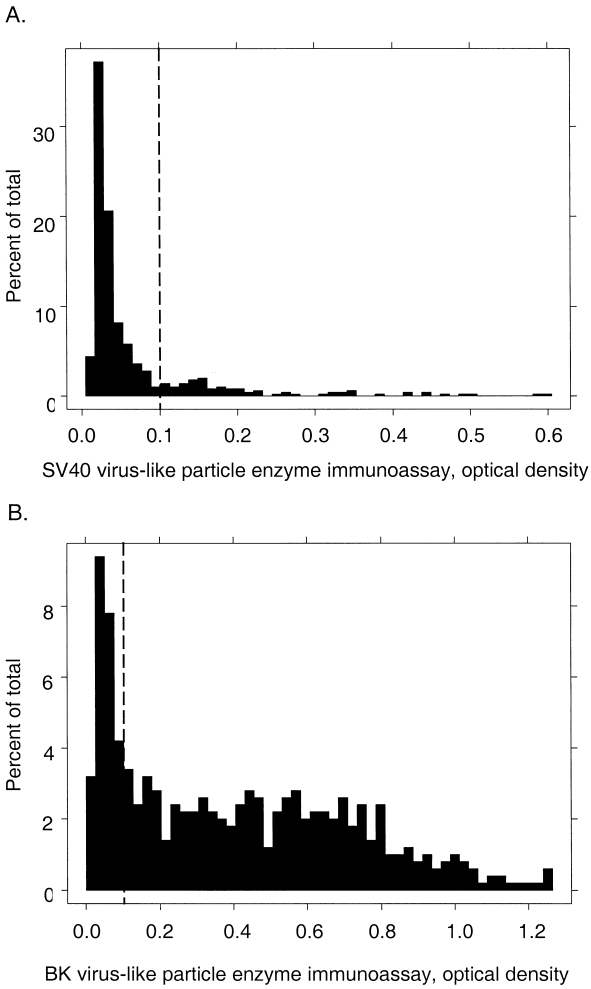

FIGURE 2. Results of polyomavirus virus-like particle enzyme immunoassay for 500 paired serum samples obtained from mothers of 250 cancer cases and controls enrolled in the Collaborative Perinatal Project in 1959–1966. Histograms of the results are shown separately for simian virus 40 (SV40) (panel A) and BK virus (panel B). Results are presented as geometric means of two duplicate absorbance (optical density) measurements. The dashed vertical line (0.10 absorbance units) represents the cutoff point for the assay. Note that the horizontal and vertical scales differ in the two different panels.

(9). One reason for this difference was that the previous investigators evaluated risk related to all inactivated poliovirus vaccines, regardless of year of receipt, whereas we considered pre-1963 vaccine to be relevant to an analysis of SV40-related cancer risk. Specifically, for children under 4 years of age, they classified one child with astrocytoma as having maternal vaccine exposure, although the mother received inactivated poliovirus vaccine only in 1963. Substantial attenuation also resulted from our inclusion of six additional neural tumors among children aged 0–3 years (table 1). We do not know why Heinonen et al. were unaware of these cases, but all were children who lacked maternal exposure to pre-1963 poliovirus vaccine. Additionally, among 631 children whose mothers received pre-1963 oral poliovirus vaccine, classified by us but not by Heinonen et al. as having relevant vaccine exposure, none developed a neural tumor. Indeed, we found only a modest association between maternal receipt of pre-1963 poliovirus vaccine and risk of neural tumors in children aged 0–3 years (hazard ratio = 1.7). Of further note, among children aged 0–7 years, the association between maternal receipt of pre-1963 vaccine and risk of neural tumors was especially strong for neuroblastoma (hazard ratio = 8.2), even though SV40 has never been detected in neuroblastoma (3, 25). Overall, the validity of our methods for ascertaining cancer outcomes was supported by the close correspondence of the observed number of cases with expected rates (13).

In our case-control study, we found no consistent relation between maternal SV40 seroconversion during pregnancy and cancer in children. With the VLP assay, maternal seroconversion was associated with an increased overall cancer risk in children (OR_{SA} = 4.0), but this association was not observed with the plaque assay (OR_{SA} = 0.9). Importantly, the few SV40 seroconversions that we observed were in mothers of children with diverse malignancies, with no apparent pattern (table 5). Seroconversion in both assays was seen for only one woman whose child developed neuroblastoma. SV40 DNA has been reported by some laboratories in cases of ependymoma, astrocytoma/glioma, Wilms' tumor, and non-Hodgkin's lymphoma (3, 26–28). Additionally, Farwell et al. previously reported an association between maternal poliovirus vaccination during pregnancy and childhood medulloblastoma (29). Even though our study included children with each of these malignancies, maternal seroconversion by either SV40 assay was documented only for one astrocytoma.

Perhaps surprisingly, we did not find an unambiguous association between seroconversion and receipt of pre-1963 inactivated poliovirus vaccine. With the VLP assay but not with the plaque assay, SV40 seroconversions were observed more frequently in mothers who received pre-1963 inactivated poliovirus vaccine than among other mothers. Still, SV40 seroconversions by either assay were distinctly uncommon in women who received pre-1963 inactivated poliovirus vaccine. SV40 neutralizing antibodies can be detected within 2–5 weeks of human or macaque infection (30–32), so our use of a 6-week lag in defining informativeness should have enabled us to detect seroconversion following receipt of pre-1963 inactivated poliovirus vaccine. Furthermore, few women were SV40-seroprevalent at the start of pregnancy (table 4), though many would have received pre-1963 inactivated poliovirus vaccine earlier in life. The proportion of persons who seroconverted to SV40 following one or more doses of pre-1963 inactivated poliovirus vaccine varied widely (8–92 percent) in previous studies (10, 33–35). These prior data and our results indicate that SV40 contamination of the inactivated poliovirus vaccine used in the United States was somewhat uneven, with varying frequencies or levels of contamination across vaccine lots (1).

The generally low amount of SV40 antibody (measured in terms of VLP absorbance value or plaque assay titer) in seropositive mothers further militates against SV40's being

TABLE 4. Distribution of mothers of cancer cases and controls enrolled in the Collaborative Perinatal Project in 1959–1966, by simian virus 40 serostatus

	Simian virus 40 virus-like particle assay					Simian virus 40 plaque neutralization assay*				
	No. of subjects	SC†	SP†	SN†	SR†	No. of subjects	SC	SP	SN	SR
Case mothers	50	4 (8)‡	6 (12)	40 (80)	0	46	3 (7)	2 (4)	40 (87)	1 (2)
Received pre-1963 vaccine	20	2 (10)	2 (10)	16 (80)	0	18	2 (11)	0	16 (89)	0
Pre-1963 IPV†	20	2 (10)	2 (10)	16 (80)	0	18	2 (11)	0	16 (89)	0
Pre-1963 OPV†	0	0	0	0	0	0	0	0	0	0
Did not receive pre-1963 vaccine	30	2 (7)	4 (13)	24 (80)	0	28	1 (4)	2 (7)	24 (86)	1 (4)
1963+ IPV	1	0	0	1 (100)	0	1	0	0	1 (100)	0
1963+ OPV	5	0	1 (20)	4 (80)	0	5	0	0	5 (100)	0
No vaccine	24	2 (8)	3 (13)	19 (79)	0	22	1 (5)	2 (9)	18 (82)	1 (5)
Control mothers	200	6 (3)	26 (13)	160 (80)	8 (4)	187	8 (4)	13 (7)	163 (87)	3 (2)
Received pre-1963 vaccine	100	5 (5)	9 (9)	82 (82)	4 (4)	94	4 (4)	5 (5)	83 (88)	2 (2)
Pre-1963 IPV	90	4 (4)	8 (9)	75 (83)	3 (3)	84	4 (5)	5 (6)	74 (88)	1 (1)
Pre-1963 OPV	10	1 (10)	1 (10)	7 (70)	1 (10)	10	0	0	9 (90)	1 (10)
Did not receive pre-1963 vaccine	100	1 (1)	17 (17)	78 (78)	4 (4)	93	4 (4)	8 (9)	80 (86)	1 (1)
1963+ IPV	45	1 (2)	7 (16)	36 (80)	1 (2)	40	1 (3)	1 (3)	38 (95)	0
1963+ OPV	5	0	2 (40)	3 (60)	0	5	0	2 (40)	3 (60)	0
No vaccine	50	0	8 (16)	39 (78)	3 (6)	48	3 (6)	5 (10)	39 (81)	1 (2)

* Plaque assay results were missing for 17 subjects.

† SC, seroconverter; SP, seroprevalent; SN, seronegative; SR, seroreverter; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine.

‡ Numbers in parentheses, percentage.

TABLE 5. Characterization of cancer cases enrolled in the Collaborative Perinatal Project in 1959–1966 whose mothers demonstrated simian virus 40 seroconversion during pregnancy

Diagnosis	Age of case child (years)	Polyomavirus serostatus of mother during pregnancy*						Vaccination of mother during pregnancy	
		SV40† VLP† assay		SV40 plaque assay		BK virus VLP assay			
		Serostatus	Absorbance values	Serostatus	Titers (reciprocal dilution)	Serostatus	Absorbance values	Status	Informativeness of paired serum samples‡
Neural tumors									
Neuroblastoma	4.9	SC†	0.06, 0.14	SC	10, 160	SP†	0.93, 1.25	Pre-1963 IPV†	Uninformative
Neuroblastoma	2.5	SC	0.06, 0.18	SP	40, 160	SP	0.40, 0.75	No vaccine	NA†
Astrocytoma	6.3	SC	0.07, 0.14	SN†	Negative, negative	SP	0.99, 0.51	No vaccine	NA
Hematologic malignancies									
Leukemia	1.8	SC	0.06, 0.12	SN	Negative, negative	SP	0.23, 0.27	Pre-1963 IPV	Informative
Leukemia	2.4	SN	0.03, 0.02	SC	10, 40	SN	0.03, 0.01	Pre-1963 IPV	Informative
Miscellaneous tumors									
Fibrosarcoma	4.7	SN	0.03, 0.08	SC	Negative, 40	SP	0.49, 0.92	No vaccine	NA

* Test results for paired maternal specimens are presented.

† SV40, simian virus 40; VLP, virus-like particle; SC, seroconverter; SP, seroprevalent; IPV, inactivated poliovirus vaccine; NA, not applicable; SN, seronegative.

‡ For women who received poliovirus vaccine during pregnancy, informative serum pairs were those in which at least one dose of poliovirus vaccine was received in the window defined by the serum dates; see Materials and Methods.

a cause of childhood cancer. Following initial SV40 infection in macaques, replication of SV40 leads to a robust antibody response (31, 32). Therefore, our results point to an absence or near absence of productive SV40 infections among mothers in the Collaborative Perinatal Project. If vaccine-related exposures in mothers did not lead to productive infection, transmission of SV40 to children, either in utero or neonatally, would have been unlikely.

The lack of a more frequent or robust immune response against SV40 in our subjects was not likely to have been due to poor assay sensitivity. Although the sensitivity of these assays for human SV40 infection is uncertain, both SV40 assays reliably detect infection in rhesus macaques, the natural host (17, 36), and the plaque assay detected SV40 seroconversion following vaccination in prior studies (33–35). Nonetheless, among mothers in our study, the results of the SV40 VLP assay and the results of the plaque assay were not highly correlated. In comparison, BK VLP reactivity was much stronger (figure 2), and results from each SV40 antibody assay were somewhat correlated with BK virus results. Our findings and those of previous investigations (17–19, 37) suggest that some low-level antibody responses to SV40 seen in humans are nonspecific and are due to cross-reactive antibodies, perhaps to BK virus. BK virus seroprevalence in Project mothers was similar to that seen in other pregnant women (38), arguing against deterioration of stored serum samples.

An important limitation of our study was the composite nature of the cancer outcomes that we evaluated. For instance, although we found an association between maternal exposure to pre-1963 inactivated poliovirus vaccine and neural tumors, the most common type of neural tumor was neuroblastoma, which has not been linked to SV40 (3). Leukemia was the most frequent hematologic malignancy, yet SV40 DNA sequences have not been detected in leukemia specimens obtained from children (39). Given the somewhat small number of children with cancer, we were precluded from reaching firm conclusions regarding particular tumor types. Additionally, it would have been interesting to examine serum samples or tumor specimens obtained from children at the time that they developed their malignancies, but these specimens were unavailable.

If vertically transmitted SV40 infection does not explain the excess cancer risk in children whose mothers received pre-1963 inactivated poliovirus vaccine during pregnancy, what accounts for this observed association? Transmission of poliovirus itself is unlikely to explain our findings, since poliovirus vaccine used in 1963+ was not linked to an increased risk of cancer (figure 1), even though a substantial proportion of this vaccine was oral poliovirus vaccine (which, unlike inactivated poliovirus vaccine, is a live vaccine). In addition, poliomyelitis survivors are not at increased risk of neural or hematologic malignancies (40). Conceivably, confounding could explain the association between maternal poliovirus vaccination and childhood cancer. For example, changes in cancer incidence over calendar time unrelated to poliovirus vaccination may play a role. Indeed, overall cancer incidence was higher in children born before 1963 than in those born subsequently (20 per 100,000 person-years vs. 11 per 100,000 person-years), and the hazard ratios reported in table 3 were somewhat attenuated after adjustment for calendar year of birth (data not shown). Alternatively, the associations with maternal vaccination might represent confounding by socioeconomic status—that is, early poliovirus vaccine may have been most widely available to women with relatively high socioeconomic status, who were then more likely to return for follow-up visits when childhood cancers were recorded. Finally, the associations between maternal vaccination and childhood cancer could have been due to chance, although the confidence intervals and related *p* values argue against this possibility (table 3).

In summary, our results do not support a link between SV40 infection during pregnancy and cancer risk in subsequently born children. Our study expands on prior work by Heinonen et al. (9) and Rosa et al. (10), including additional children with cancer and incorporating state-of-the-art serologic assays. Nonetheless, our conclusion regarding maternal vaccination and childhood cancer in the Collaborative Perinatal Project cohort remains the same as that reached by Rosa et al.: "there appears to be an association between the administration of killed-poliovirus vaccine to mothers and neurologic [and hematologic] tumors in their offspring that is not due to SV40; its basis remains to be clarified" (10, p. 1469). Additional serology-based case-control studies may be useful in elucidating the potential role of SV40 in cancer.

ACKNOWLEDGMENTS

The authors thank Dr. Robert J. Biggar of the National Cancer Institute for helpful comments.

REFERENCES

1. Shah K, Nathanson N. Human exposure to SV40: review and comment. Am J Epidemiol 1976;103:1–12.
2. Institute of Medicine, National Academy of Sciences. Immunization safety review: SV40 contamination of polio vaccine and cancer. Washington, DC: National Academy Press, 2002.
3. Bergsagel DJ, Finegold MJ, Butel JS, et al. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N Engl J Med 1992;326:988–93.
4. Engels EA, Sarkar C, Daniel RW, et al. Absence of simian virus 40 in human brain tumors from northern India. Int J Cancer 2002;101:348–52.
5. Girardi AJ, Sweet BH, Hilleman MR. Factors influencing tumor induction in hamsters by vacuolating virus, SV40. Proc Soc Exp Biol Med 1963;112:662–7.
6. Engels EA, Katki HA, Nielsen NM, et al. Cancer incidence in Denmark following exposure to poliovirus vaccine contaminated with simian virus 40. J Natl Cancer Inst 2003;95:532–9.
7. Carroll-Pankhurst C, Engels EA, Strickler HD, et al. Thirty-five year mortality following receipt of SV40-contaminated polio vaccine during the neonatal period. Br J Cancer 2001;85:1295–7.
8. Strickler HD, Rosenberg PS, Devesa SS, et al. Contamination of poliovirus vaccines with simian virus 40 (1955–1963) and subsequent cancer rates. JAMA 1998;279:292–5.

9. Heinonen OP, Shapiro S, Monson RR, et al. Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. Int J Epidemiol 1973;2:229–35.
10. Rosa FW, Sever JL, Madden DL. Absence of antibody response to simian virus 40 after inoculation with killed-poliovirus vaccine of mothers of offspring with neurologic tumors. (Letter). N Engl J Med 1988;318:1469.
11. Niswander KR, Gordon M, eds. The women and their pregnancies. Philadelphia, PA: W B Saunders Company, 1972.
12. Read JS, Clemens JD, Klebanoff MA. Moderate low birth weight and infectious disease mortality during infancy and childhood. Am J Epidemiol 1994;140:721–33.
13. Klebanoff MA, Read JS, Mills JL, et al. The risk of childhood cancer after neonatal exposure to vitamin K. N Engl J Med 1993;329:905–8.
14. Shiono PH, Chung CS, Myrianthopoulos NC. Preconception radiation, intrauterine diagnostic radiation, and childhood neoplasia. J Natl Cancer Inst 1980;65:681–6.
15. Wei LJ, Lin DY, Weissfeld L. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. J Am Stat Assoc 1989;84:1065–73.
16. Rothman KJ, Greenland S. Case-control studies. In: Rothman KJ, Greenland S, eds. Modern epidemiology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 1998:93–114.
17. Viscidi RP, Rollison DE, Viscidi E, et al. Serological cross-reactivities between antibodies to simian virus 40, BK virus, and JC virus assessed by virus-like-particle-based enzyme immunoassays. Clin Diagn Lab Immunol 2003;10:278–85.
18. Carter JJ, Madeleine MM, Wipf GC, et al. Lack of serological evidence for prevalent simian virus 40 infection in humans. J Natl Cancer Inst 2003;95:1522–30.
19. de Sanjose S, Shah KV, Domingo-Domenech E, et al. Lack of serological evidence for an association between simian virus 40 and lymphoma. Int J Cancer 2003;104:522–4.
20. Strickler HD, Goedert JJ, Fleming M, et al. Simian virus 40 and pleural mesothelioma in humans. Cancer Epidemiol Biomarkers Prev 1996;5:473–5.
21. Breslow NE, Chatterjee N. Design and analysis of two-phase studies with binary outcome applied to Wilms tumour prognosis. Appl Stat 1999;48:457–68.
22. Breslow NE, Cain KC. Logistic regression for two-stage case-control data. Biometrika 1988;75:11–20.
23. Chen J. Semiparametric efficient and inefficient estimation for the auxiliary outcome problem with the conditional mean model. (Doctoral dissertation). Seattle, WA: University of Washington, 2002.
24. Ikeda Y, Lister J, Bouton JM, et al. Congenital neuroblastoma, neuroblastoma in situ, and the normal fetal development of the adrenal. J Pediatr Surg 1981;16(suppl 1):636–44.
25. Martini F, Iaccheri L, Lazzarin L, et al. SV40 early region and large T antigen in human brain tumors, peripheral blood cells, and sperm fluids from healthy individuals. Cancer Res 1996; 56:4820–5.
26. Martini F, Lazzarin L, Iaccheri L, et al. Different simian virus 40 genomic regions and sequences homologous with SV40 T antigen in DNA of human brain and bone tumors and of leukocytes from blood donors. Cancer 2002;94:1037–48.
27. Vilchez RA, Madden CR, Kozinetz CA, et al. Association between simian virus 40 and non-Hodgkin lymphoma. Lancet 2002;359:817–23.
28. Shivapurkar N, Harada K, Reddy J, et al. Presence of simian virus 40 DNA sequences in human lymphomas. Lancet 2002; 359:851–2.
29. Farwell JR, Dohrmann GJ, Marrett LD, et al. Effect of SV40 virus-contaminated polio vaccine on the incidence and type of CNS neoplasms in children: a population-based study. Trans Am Neurol Assoc 1979;104:261–4.
30. Morris JA, Johnson KM, Aulisio CG, et al. Clinical and serologic responses in volunteers given vacuolating virus (SV40) by respiratory route. Proc Soc Exp Biol Med 1961;108:56–9.
31. Shah KV, Willard S, Meyers RE, et al. Experimental infection of rhesus with simian virus 40 (SV40). Proc Soc Exp Biol Med 1969;130:196–203.
32. Minor P, Pipkin PA, Cutler K, et al. Natural infection and transmission of SV40. Virology 2003;314:403–9.
33. Magrath DI, Russell K, Tobin JO. Vacuolating agent. BMJ 1961;2:287–8.
34. Sweet BH, Hilleman MR. The vacuolating virus, SV40. Proc Soc Exp Biol Med 1960;105:420–7.
35. Shah KV, McCrumb FR, Daniel RW, et al. Serological evidence for a simian-virus-40-like infection of man. J Natl Cancer Inst 1972;48:557–61.
36. Meyer HM Jr, Hopps HE, Rogers NG, et al. Studies on simian virus 40. J Immunol 1962;88:796–806.
37. Brown P, Tsai T, Gajdusek DC. Seroepidemiology of human papovaviruses: discovery of virgin populations and some unusual patterns of antibody prevalence among remote peoples of the world. Am J Epidemiol 1975;102:331–40.
38. Stolt A, Sasnauskas K, Koskela P, et al. Seroepidemiology of the human polyomaviruses. J Gen Virol 2003;84:1499–504.
39. Smith MA, Strickler HD, Granovsky M, et al. Investigation of leukemia cells from children with common acute lymphoblastic leukemia for genomic sequences of the primate polyomaviruses JC virus, BK virus, and simian virus 40. Med Pediatr Oncol 1999;33:441–3.
40. Nielsen NM, Wohlfarht J, Aaby P, et al. Cancer risk in a cohort of polio patients. Int J Cancer 2001;92:605–8.